Pdot-2 is PFBT/TMOS/TEOS=2/1/1; Pot-3 is PFBT/TMOS/TEOS=2/2/2; Pdot-4 is PFBT/TCOS/TEOS=2/1/1; Pdot-5 is PFBT/TMOS/TEOS=2/2/2; —N indicates negative controls, where cells were not incubated with biotinylated primary antibody, and were directly incubated with hybrid polymer dot-streptavidin conjugates without biotinylated primary antibody; and —P indicates positive labeling. The results indicate that the hybrid polymer dots exhibit similar or slightly higher cell-labeling brightness as compared to the polymer dots functionalized by the PS-PEG-COOH blending method, the bare polymer dots.

Example 10

Photostability of the Hybrid Polymer Dots for Cell Labeling

[0232] This example demonstrates photostability measurements of the cells labeled with hybrid polymer dot bioconjugates.

[0233] Hybrid polymer dots were prepared according to Example 1 to make hybrid polymer dots using PFBT, TMOS, and TEOS at ratios of 2:1:1 and 2:2:2, as well as hybrid polymer dots using PFBT, TCOS, and TEOS at ratios of 2:1:1 and 2:2:2.

[0234] MCF-7 cells were labeled as provided in Example 8. The hybrid polymer dot bioconjugate labeled cells were imaged on a fluorescence confocal microscope (Zeiss LSM 510). For photobleaching studies, confocal fluorescence images were recorded continuously for the cells labeled with the hybrid polymer dots and those labeled with the polymer dots blended with PS-PEG-COOH. Photobleaching data points were extracted by analyzing the fluorescence images using a custom-coded Matlab program. As shown in FIG. **7** and FIG. **9**, photobleaching curves extracted from the fluorescence images indicate that the hybrid polymer dot were more photostable than the polymer dots functionalized by the PS-PEG-COOH blending method.

Example 11

Gel Electrophoresis of Hybrid Polymer Dots and Related Bioconjugates

[0235] This example demonstrates the characterization of the functional groups on the surface of the hybrid polymer dots using gel electrophoresis.

[0236] Gel electrophoresis was performed using a 0.7% agarose gel. Agarose gel electrophoresis of functionalized hybrid polymer dots was carried out using a Mupid®-exU submarine electrophoresis system. The functionalized hybrid polymer dots, in 30% glycerol, were loaded onto a 0.7% agarose gel containing 0.1% polyethylene glycol. The functionalized hybrid polymer dot-loaded gel was run for 20 min at 135 V in tris-borate-EDTA (TBE) buffer, and then imaged on a Kodak image station 440CF system. As shown in FIG. **10**, compared to unfunctionalized, bare polymer dots, the functionalized hybrid polymer dots exhibited an increase in mobility in the gel. Notably, once the hybrid polymer dots are conjugated to streptavidin, the hybrid polymer dot-streptavidin bioconjugates show decreased mobility. This can be used to detect successful bioconjugation.

Example 12

Determination of Network Structure for Hybrid Polymer Dots

[0237] This example demonstrates the characterization of the interpenetrated network generated in formation of the hybrid polymer dots utilizing TEM and flow cytometry.

[0238] Interpenetrated hybrid polymer dots were prepared as according to Example 1 using PFBT, TCOS, and TEOS, at a weight ratio of 1:1:1.

[0239] PFBT-14% C_2COOH, a functionalized chromophoric polymer, was dissolved in tetrahydrofuran (THF) by stirring under inert atmosphere to make a solution with concentration of 1 mg/mL. TCOS, an organic silane, was dissolved in THF to make a solution with concentration of 1 mg/mL. TEOS was dissolved in THF to make a solution with concentration of 1 mg/mL. The above solutions of PFBT-14% C_2COOH, TCOS, and TEOS were diluted into THF to form 2 mL of a mixed homogenous solution containing PFBT-14% C_2COOH at a concentration of 0.1 mg/mL. Deionized water was obtained and the pH value of it was adjusted to approximately 11. The 2 mL quantity of the PFBT-14% C_2COOH solution was quickly added to 10 mL of the aqueous solution while sonicating the mixture. THF was removed by nitrogen stripping, and the solution was concentrated by continuous nitrogen stripping to 2 mL on a hotplate at 90° C., which was followed by filtration through a 0.2 micron filter. This afforded hybrid polymer dots wherein the chromophoric polymer was directly functionalized with carboxyl groups, resulting in Pdots not interpenetrated with Silane-COONa.

[0240] FIG. **15** provides chemical structures of the chromophoric polymer polyfluorene-benzothiadiazole PFBT-14% C_2COOH, as well as organic silane molecules such as TCOS and TEOS. A resultant polymer dot directly functionalized with carboxyl is also illustrated in FIG. **15**.

[0241] Hybrid polymer dot-streptavidin bioconjugates were prepared as according to Example 8 to make PFBT-14% C_2COOH polymer dot-streptavidin bioconjugates as well as Silane-COONa polymer dot-streptavidin bioconjugates.

[0242] Flow cytometry was used to evaluate the labeling brightness of the hybrid polymer dot-streptavidin bioconjugates, as according to Example 8. FIG. **16** shows flow cytometry results of MCF-7 cells labeled with the PFBT-14% C_2COOH hybrid polymer dots or labeled with the Silane-COONa hybrid polymer dots. "Negative of" indicates control cells incubated with hybrid polymer dot-streptavidin bioconjugates in the absence of biotinylated primary antibody. "Positive of" indicates cells incubated with the hybrid polymer dot-strepdavidin bioconjugates and biotinylated primary antibody. Fluorescence was observed for both of the "positive" groups, indicating the specific binding of streptavidin to carboxyl functionality applied to both types of Pdots generated. The result indicated that the external carboxyl availability of PFBT-14% C_2COOH hybrid polymer dots is similar to the external carboxyl availability of Silane-COONa hybrid polymer dots. This result indicated that the short carboxylic acid functional group of the PFBT backbone chain inside the Pdots is not encased by the silica network as a shell outside the hybrid Pdots, but instead exists as a part of an interpenetrated network formed between the polymer chains and silica network. The result of this flow cytometry experiment